Is the life-cycle of the European pine sawfly constrained to a phenological window of host susceptibility?

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Abstract

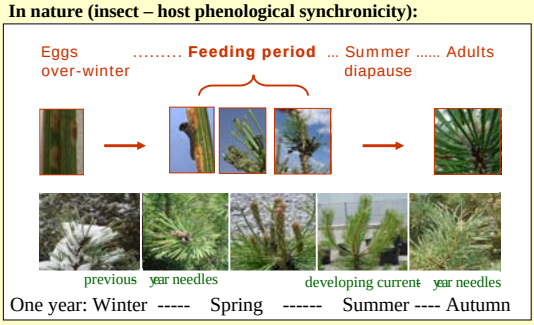
We tested the hypothesis that the life cycle of European pine sawfly is constrained to a narrow temporal window in early spring by phenological changes in plant quality that results in a limited period of host suitability and precludes an apparent opportunity for a second generation. The phenology of the European pine sawfly was experimentally advanced, delayed, or not manipulated relative to phenological stages of its host, Scots pine. Consistent with the predictions of the phenological window hypothesis, larval growth and survival progressively decreased as neonate larvae fed on earlier or more advanced needle ages. No neonate survived on previous-year needles in mid June nor in mid August. In September, host suitability slightly increased, but it was still very low, as indicated by highly reduced larval growth and survival. The suitability of new foliage subsequently increased in late winter to become highly suitable the following spring. Monoterpene level progressively increased in current –year needles, and remained constant during the next year. Chemical analyses detected a phenolic-based compound that was present at a higher concentration on the younger foliage.

Introduction

Plants are consumed by some insects only at specific periods of the season, and at a particular phenological stage of development. This period, or window of host suitability, is expected to be of best quality for the herbivore (Feeny 1976, Mattson et al. 1982).

Pine trees hold their foliage all year, thus being potential target for herbivores at any time; however the European pine sawfly (*Neodiprion sertifer*), a destructive insect pest of pine, only feeds during a narrow "window" of time in early spring (Fig. 1).

In nature (insect – host phenological synchronicity):



Scots pine undergoes a single and synchronized flush-growth per growing season. Source/sink processes may modulate resource allocation between growth and defense.

Pine flush-growth is partially supported by the allocation of reserves stored in the previous-year needles. This process may progressively decrease previous-year-needle suitability for *N. sertifer*. Simultaneously, developing structures may be protected by increased abundance of allocated secondary chemicals.

Fig. 1. Univoltine life-cycle of the European pine sawfly (*N. sertifer*) in relation to host phenology. *N. sertifer* neonates emerge in mid April and feed only on previous-year needles.

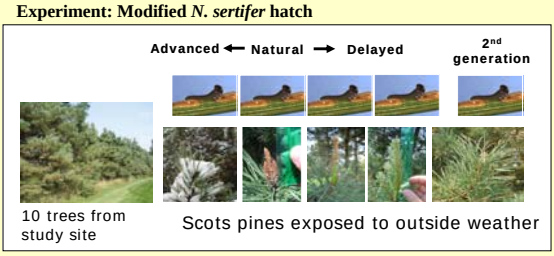
Hypothesis: The EPSF life-cycle is restricted to an early season (April-May) host phenological window of susceptibility. A host phenological window of resistance constrains the evolution of a second generation.

Objectives

- **Objective 1:** Manipulate insect/host phenological synchronicity; advancing, delaying or not manipulating insect phenology relative to phenological stages of naturally growing Scots pines, and quantify *N. sertifer* neonate growth and survival.
- **Objective 2:** Quantify the phenological variation in Scots pine phytochemistry, relating it to *N. sertifer* growth and survival.

Research Methods

Experiment: Modified *N. sertifer* hatch



Bioassays were repeated over time as pine phenology advanced. Each date, a group of 10 neonates were fed last-year- and current-year-needles, the later when available. (20°C, 15hDL).

***N. sertifer* neonates:**

A) Eggs were kept quiescent until needed (1±1°C). Prolonged cold storage for up to 90 days did not affect the life-span of food-deprived neonates (Fig. 2) suggesting that embryonic reserves (e.g. vitellin) were not significantly depleted during extended quiescence. Egg-bearing needles were clipped 10 days before bioassay starting date and placed at 20°C to stimulate hatch.

B) For the late-season bioassays (September), a 2nd generation of larvae was reared (Sullivan and Wallace 1967, Baldassari et al. 2003).

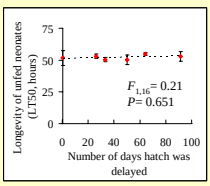


Fig. 2. Effect of prolonged cold storage of quiescent eggs on the longevity of unfed neonates. (LT50: time for 50% of the neonates to die).

Results and Discussion

In 2006, *N. sertifer* natural hatch occurred in synchrony with needles of 333 and 697 days-old, modifying host-insect synchronicity decreased neonate growth and survival.

An artificially reared 2nd generation of larvae hatched in September when current-year needles (115 days-old), and previous-year needles (480 days-old) were available. Larval growth and survival were much lower on these age-class needles. Interestingly, these larvae emerged over a longer period of time (15 days) than the 1st natural generation (5 days), which desynchronized larval stages (not shown).

N. sertifer natural hatch matches a temporal window of foliage suitability. Current year needles are initially unsuitable, then become suitable by March of the next year, then become unsuitable during the summer, and subsequently become suitable again during the next spring.

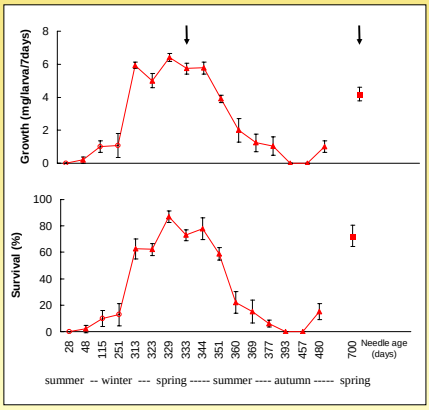


Fig. 3. *N. sertifer* neonate growth and survival after advanced, not modified, and delayed hatch, in relation to needle age. Needle age is the no. days since visible (>1mm) in May 15th of: ■ 2004, ▲ 2005, or ○2006, until bioassay date. Arrow indicates naturally available needles when *N. sertifer* hatched in 2006.

Source / sink processes decreasing needle suitability

The decline in needle suitability (needles ageing from 333 to 390 days) corresponded with the expected allocation of reserves towards current-year growth. As needles aged, specific needle mass decreased (linear regression: $F_{1,28} = 7.63$, $P = 0.01$) (Fig. 4). Concordantly, growth of 1st instar cohorts declined as pine phenology advanced (Fig. 5).

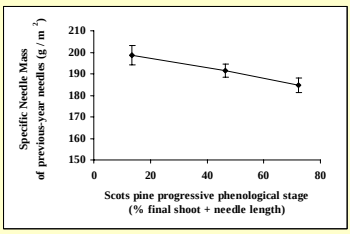


Fig. 4. Effect of progressive development of shoot and current- year foliage over needle specific mass.

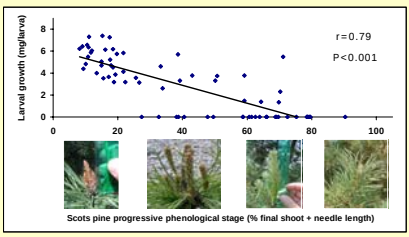


Fig. 5. Relationship between larval growth and progressive pine phenology. Larvae fed with last year needles. Current- year's shoot and needle length were added to conform a progressive measurement of ontogeny (Sampson et al. 2003).

As needle suitability declined, apparent digestibility (AD), and the efficiency of conversion of ingested food (ECI) decreased, while consumption rate and the efficiency of conversion of digested food (ECD) were not affected (Table 1).

Table 1. Food utilization efficiency. Determined for 5 just molted 3rd instar per 10 trees. Larvae fed for 48h with previous- year needles collected in April 19th (339 days old), May 12th (362 days old), and June 5th (386 days old) of 2006.

Needle age (days)	RGR Relative Growth Rate (mg/mg/day)	RCR Relative Consumption Rate (mg/mg/day)	AD Apparent Digestibility (%)	ECD Efficiency of Conversion of Digested Food (%)	ECI Efficiency of Conversion of Ingested Food (%)
339	0.478	2.94	29.42	56.57	16.51
362	0.399	3.25	24.21	52.67	12.15
386	0.334	3.18	20.66	53.42	10.40
P value	*0.001*	*0.385*	*0.005*	*0.869*	*<0.001*

Phytochemistry

Monoterpene concentration increased in the current-year needles in relation with needle age (Fig 6, needle ages of 33 to 94). Total monoterpenes remained comparatively stable in more mature needles. Needle quality (i.e. nitrogen, and carbohydrates) has been reported to decrease in the old needles while the shoot expands (Watt 1987, Ericsson 1979), this change together with high levels of defensive compounds are perhaps a combination of factors that limit the survival of this species.

A phenolic glycoside and its derivatives were found at higher concentration on the new needles decreasing as needles matured. Compound identity is being confirmed by NMR spectroscopy in collaboration with Dr. A. Eyles and Dr. W. Jones. Perhaps, this phenolic- based compound plays a role on the life-history strategy of *N. sertifer* and Scots pine. This chemical shows a pattern of seasonal decay that is similar to a feeding deterrent previously described for Scots pine needles (podocarpenoic acid: Niemelä et al. 1982).

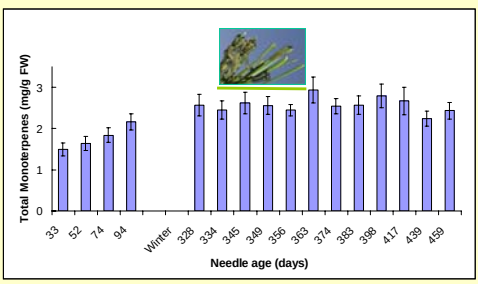


Fig. 6. Dynamics of total monoterpenes (alpha pinene, beta pinene, camphene, and myrcene) as 2005 needles aged. (GC- FID)

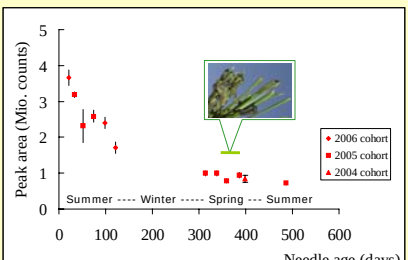


Fig. 7. Dynamics of a phenolic glycoside and its derivative in relation to needle age. (HPLC)

Conclusions

- Modifying insect – host synchronicity had an enormous deleterious effect on *N. sertifer* growth and survival.
- A host phenological window of resistance highly constrained growth and survival of *N. sertifer* 2nd generation larvae.
- Growth and survival of 1st instar larvae declined as pine phenology advanced. Needle suitability changed rapidly and in relation to the developmental stage of current-year growth.
- Further studies on host defensive chemistry and nutritional value will increase our understanding of source/sink processes modulating phenological changes in host suitability.

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